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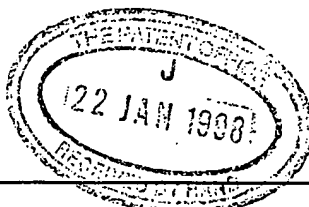
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Dated 7th January 1999

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## Request for grant of a patent

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1. Your reference

F7414(V)

2. Patent application number  
(The Patent Office will fill in this part)

**9801410.3**

**22 JAN 1998**

3. Full name, address and postcode of the or of each applicant (underline all surnames)

UNILEVER PLC

UNILEVER HOUSE, BLACKFRIARS  
LONDON, EC4P 4BQ

Patents ADP number (if you know it)

1628002

If the applicant is a corporate body, give the country/state of its incorporation

UNITED KINGDOM

4. Title of the invention

FROZEN FOOD PRODUCT

5. Name of your agent (if you have one)

KIRSCH, Susan Edith

"Address for Service" in the United Kingdom to which all correspondence should be sent (including the postcode)

PATENT DIVISION, UNILEVER PLC  
COLWORTH HOUSE, SHARNBROOK  
BEDFORD, MK44 1LQ

Patents ADP number (if you know it)

5991054002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number  
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Date of filing  
(day/month/year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing  
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8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

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Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77) 1

Request for substantive examination (Patents Form 10/77)

Any other documents (please specify)

11. I/We request the grant of a patent on the basis of this application.

Signature(s)

Date: 22 January 1998

Sandra EDWARDS, Authorised Signatory

12. Name and daytime telephone number of person to contact in the United Kingdom Petra Kimber 01234 222893

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Frozen Food productTechnical Field of the Invention

5 The invention relates to food products containing Anti-freeze peptides (AFPs), in particular to frozen food products containing AFPs.

Background to the Invention

10

Anti-freeze peptides (AFPs) have been suggested for improving the freezing tolerance of foodstuffs. In particular it has been suggested that some AFPs may be capable of increasing the smooth texture of frozen food  
15 products such as ice cream. Up till now, however the use of AFPs has not been applied to commercially available food products. One reason for this is that up till now it has proved difficult to reproducibly produce a frozen food product having the desired texture and eating  
20 characteristics.

Applicants believe that one of the possible reasons for the lack of desired texture in frozen food products containing AFP is that although the AFP is capable of  
25 recrystallisation inhibition it is often not capable of avoiding less favourable textures. Applicants believe that one of the explanations for this is that AFPs seem capable of controlling the growth of the ice-crystals. However the presence of AFP may also lead to an adverse effect in that  
30 the texture of the product is unfavourably affected during the manufacturing process.

The present invention therefore aims at defining manufacturing conditions which improve the texture of AFP containing frozen food products.

5 Surprisingly it has now been found that if the conditions for producing the frozen food material are carefully chosen, this leads to an improved texture. In particular applicants have found that if the production process involves the use of a screw extruder or a (piston)  
10 compactor, this leads to more favourable textures of the frozen product, said texture being maintained for prolonged storage periods.

Accordingly in a first aspect the present invention relates  
15 to a process for the manufacture of a frozen food product comprising AFPs, wherein a screw extruder or (piston) compactor is used.

#### Background to the invention

20

For the purpose of this inventions the term AFP has the meaning such as well-known in the art, see for example "Antifreeze proteins and their potential use in frozen food products", Marilyn Griffith and K. Vanya Ewart,  
25 Biotechnology Advances, Vol 13, pp 375-402, 1995.

Anti-freeze peptides have been described in various literature places. Also these literature places suggest their use in food products, but normally no actual  
30 indications are given how to prepare food products of good quality on an industrial scale.

WO 90/13571 discloses antifreeze peptides produced chemically or by recombinant DNA techniques from plants. The AFPs can suitably be used in food-products such as ice cream. Again no guidelines are given as to how to obtain  
5 smooth textures.

WO 92/22581 discloses AFPs from plants, which can be used for controlling ice crystal growth. This document also describes a process for extracting a polypeptide  
10 composition from intercellular spaces of plants by infiltrating leaves with an extraction medium without rupturing the plant cells.

In our non pre-published patent applications  
15 PCT/EP97/03634, PCT/EP97/03635, PCT/EP97/03636, PCT/EP97/03637 and PCT/EP97/06181 the use of AFPs in frozen food products such as frozen confectionery products has been described.

20 The present invention aims at providing the food manufacturer a greater flexibility of using AFP material in frozen food products when aiming to obtain a product with improved recrystallisation properties in combination with a good texture. In particular it has been found that the  
25 texture of frozen food products containing AFPs can markedly be improved by carefully controlling its production method.

The invention is based on the finding that if the frozen  
30 product is produced by a process involving a screw extruder or a compactor, this can lead to an improved texture of the product.

Traditional freezing processes for frozen products such as frozen confectionery products for example involve the use of scraped surface heat exchangers whereby the mix to be  
5 frozen is subjected to shear during the freezing process. Generally in this traditional freezing process it takes relatively long for the product to reach a temperature of -5 °C or lower.

10 Alternative freezing processes, which for example are often used for the freezing of water-ice involves the low shear or quiescent freezing of the mix, for example by filling a mould and dipping the mould into a cold bath, for example filled with brine.

15

Many other freezing processes are known e.g. batch freezing, plate freezing, drum freezing, freezing in screw extruders, instant freezing, etc.

20 The freezing of frozen confectionery product by means of a screw extruder is for example described in: EP 713,650 (Societe des Produits Nestle), EP 410,512 (HMF Krampe & Co et al); EP 561,118 (Milchhof-Eiskrem GmbH et al), EP 351,476 (Goavec S.A. Societe Dite).

25

Preferably the manufacturing process of the invention involves the use of a screw extruder whereby the extrusion temperature of the frozen product is -8 °C or lower, more preferred from -10 to -25 °C, most preferred from -12 to -

30 20 °C.

Suitable screw extruders for use in the process of the invention can for example be twin screw extruders such as described e.g. in EP 561,118. Also single screw extruders may be used. Also extruders may be used which combine more than one function of the ice-cream manufacturing process (see e.g. EP 713,650).

The conditions under which the screw extruder operates may vary e.g. depending on the type and size of the extruder used. Applicants believe that it is well within the ability of the skilled person to select those operating conditions such that a favourable quality of the product is obtained. Examples of suitable operating conditions are given in the examples.

15

Alternatively a compactor may advantageously be used in the manufacturing of frozen food products with AFPs. All suitable compactors such as presses may be used, especially preferred is the use of a piston compactors whereby pressure is applied onto the food products by means of the movement of a piston. Traditionally piston compactors have for example been used in the filling of sausages. Again--- applicants believe that it is within the ability of the skilled person to select the appropriate operating conditions of the (piston) compactor.

#### Optional pre-freezing

In some advantageous embodiments of the invention, the frozen product is at least partially pre-frozen prior to the use of the screw extruder or (piston) compactor. Applicants have found that such a pre-freezing step may



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further enhance the quality of the product. Suitable pre-freezing steps may for example involve a standard freezer such as for example described in EP 401 512. Very advantageously, however the pre-freezing involves a  
5 nucleation dominated freezing process, most preferred a drumfreezing process.

Generally in the freezing of a composition, two distinct phases can be seen: at the onset of the freezing process  
10 many small ice-crystals are rapidly formed. This phase is called the nucleation phase of the freezing process.

Following the nucleation process the remaining part of the composition freezes onto the surface of the nuclei and therewith contributes to the growth of the ice-crystals.  
15 This phase in the freezing process is called the growth phase. In a growth dominated freezing process most of the composition is frozen during the growth phase, in a nucleation dominated freezing process most of the composition is frozen during the nucleation phase.

20

Applicants believe that it is well within the ability of the skilled person to select those methods which result in a nucleation dominated pre-freezing processes.

25 For example rapid pre-freezing processes tend to be nucleation dominated. Suitable processes may for example involve:

- 30 (a) surface freezing, preferably film freezing onto a cold surface;
- (b) freezing of supercooled systems;
- (c) decompression freezing;

(d) freezing at very low temperatures.

(e) rapid particulate freezing, preferably condensation freezing.

5 Other rapid pre-freezing processes will be apparent to the skilled person and are also embraced in the scope of the present invention. Preferably the freezing processes involve no or low shear.

10 The use of a screw extruder or compactor may very advantageously applied to products which have been pre-frozen under such conditions that a particulate (partial) frozen material is produced e.g. flakes, pellets, powders, extended rods or sheets. For these pre-frozen products the  
15 use of screw extruders or (piston) compactors may advantageously lead to the compaction of the particulate material into a more solid structure.

Surface pre-freezing preferably involves the application of  
20 a thin film or discrete particles onto a cold surface, optionally followed by removal of the frozen material. Preferably the film or particle thickness is from 0.01 to 5 mm. The cold surface is preferably at a temperature below  $-15^{\circ}\text{C}$ , more preferred less than  $-20^{\circ}\text{C}$ , most preferred less  
25 than  $-25^{\circ}\text{C}$ . Suitably the surface can be cooled by applying coolants such as liquid nitrogen, glycols or methanol. The removal can be done by any suitable means, for example by scraping, therewith producing frozen flakes or pellets which can then be further processed. Obviously during  
30 further processing care should be taken to avoid substantial melting of the composition, which may result in growth dominated re-freezing.

In a very preferred embodiment surface pre-freezing involves film freezing on a drum freezer which is for example cooled with liquid nitrogen or methanol, followed 5 by removal of the film from the drum freezer. This embodiment of the invention leads to a flaky product after pre-freezing, whereby the flakes are further compacted in a screw extruder.

10 In a further preferred embodiment of surface pre-freezing a cryogenic plate freezer cooled with liquid nitrogen is used to produce frozen pellets. These pellets are then further compacted in a screw extruder.

15 Another method of achieving rapid, nucleation dominated pre-freezing is to produce a supercooled system at low temperatures followed by sudden freezing e.g. by applying a shock to the supercooled liquid. The rapid freezing of a supercooled liquid generally is a nucleation dominated 20 freezing process.

Preferably the supercooled liquid has a temperature of at least 1 degree below the melting point.

25 A third method of achieving rapid nucleation dominated pre-freezing is to use decompression pre-freezing. This involves the applying of high pressures to a liquid system while cooling it followed by removing the overpressure. This removal of the pressure then results in a rapid 30 nucleation dominated freezing.

Preferably the pressure to be applied is from 100 to 3000 bar, for example from 200 to 2000, generally from 300 to 1300 bar. The temperature of the product before removing the over-pressure is preferably at least 5 degrees below the melting point at atmospheric pressure, preferably 6-10 degrees below the melting point.

A fourth method of ensuring nucleation dominated pre-freezing is the application of very low temperatures. For example small drops of material to be frozen may be immersed into a fluid freezing medium e.g. liquid hexane or liquid nitrogen. Preferably the freezing temperature for this method is below  $-50^{\circ}\text{C}$ . This method works best for relatively small or thin products to be frozen. Small products are preferably less than 5 grams, more preferred from 0.001 to 3 grams, most preferred 0.01 to 1 gram and may for example be drops of liquid immersed in the freezing medium. Relatively thin products may for example be sheets or thin streams of products, preferably having at least 1 dimension of less than 2 cm, more preferred 0.1 to 1 cm.

The product for use in this pre-freezing method may for example be directly immersed into the freezing liquid, alternatively however the products are first contacted with a protective layer e.g. filled into a mould, pumped through a pipe whereby these are contacted with the cooling medium.

A fifth preferred method to freeze food products of the invention involves rapid particulate freezing, preferably condensation freezing. This may for example be achieved by spraying a liquid mix to be frozen into a very cold gaseous environment or into a cooled environment. An especially

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preferred method for rapidly freezing a liquid into particulates is condensation freezing. Most preferred is the use of techniques which are for example use in the production of artificial snow.

5

The production of artificial snow is widely described in the literature. Often artificial snow is produced in so-called snow-cannons whereby the water is frozen by spraying a mixture of water and pressurised air. Preferably the snow-making takes place in an environment having a temperature of less than -3 C, most preferred -5 to -50 C and a relative humidity of less than 75%, most preferred less than 50%.

15 Frozen particulates obtained by this fifth method can vary in size, but generally the number average diameter of the particles will be up to 10 mm, more preferred less than 5 mm. Generally each particulates will comprise multiple aggregated ice-crystals.

20

#### Other steps in the manufacturing process

The complete manufacturing process of the frozen products of the invention may comprise further optional steps in addition to screw extrusion or piston compaction: for example the mixing of the ingredients, ageing, pasteurization, homogenization, pre-freezing (see hereabove), pre-aeration etc. These optional steps can take place in any suitable order.

30

Applicants believe that it is within the ability of the skilled person to define the most appropriate operating

conditions of the screw extruder e.g. speed of rotation of the screw(s), pressure difference between entrance and exit of the extruder, temperature profile in the extruder etc. Examples of suitable operating conditions are mentioned in  
5 EP 713,650.

Applicants have found that the use of a screw extruder or (piston compactor) in the production of AFP containing frozen food products is very advantageous in that it can  
10 lead to very small ice-crystal sizes which can be maintained for long periods of storage. Preferably the freezing conditions are chosen such that the average particle size of the ice-crystals crystals in the final frozen product is from 0.01 to 20 micrometer, said particle  
15 sizes to be maintained in said range upon storage at  $-10^{\circ}\text{C}$  for 3 weeks. Particularly advantageous is the fact that by using a screw extruder or a (piston) compactor products may be formed which are homogeneously solid and no (fine) particulates (e.g. powders, flakes, pellets, thin rods or  
20 thin sheets). Preferably non-particulate products of the invention have a smallest dimension of more than 2 cm, more preferred more than 2.5 cm, most preferred more than 3 cm. Accordingly a further embodiment of the invention relates to a non-particulate frozen food product comprising AFPs,  
25 said product having an average ice crystal size of 0.01 to 20 micrometer, wherein said crystal size is maintained between 0.01 and 20 micrometer upon storage for 3 weeks at  $-10^{\circ}\text{C}$ .

30 After freezing the product may be further handled. For example the product may be filled into packages say of 0.05 to 10 litres and then stored. Alternatively the product may

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be further shaped or formed into the final product. For example the product can be used to the shaped into ice-cream gateaux.

5 A further advantage of the invention is that products of the invention do generally not need to be subjected to a hardening step i.e. in a hardening tunnel. This advantage has for example been suggested for AFP products in general in US 5,620.732 (Pillsbury).

10

The process as described in US 5,620,732 however has as a disadvantage that this is not suitable for the production of luxury stick products. These products are traditionally made by extruding and cutting a block of ice-cream, 15 hardening the block, followed by stick insertion and coating e.g. with chocolate or fruit water ice. If the hardening step is omitted for AFP containing products this leads to problems in the further handling e.g. during stick insertion or further coating.

20

Surprisingly applicants have found that the combined use of AFPs and screw extrusion now renders it possible to product luxury stick products without the use of a hardening step.

25 Frozen food products of the invention may be any food product which can be stored and/or eaten in the frozen state. Examples of frozen food products which may contain AFPs are processed food products such as for example frozen bakery products e.g. doughs, batters, cakes etc., frozen 30 culinary products for example soups, sauces, pizzas, frozen vegetable products such a compote, mashed potato, tomato paste etc.

Applicants have found that the method of the invention is best applicable to food systems which are fluid or liquid prior to freezing. A very preferred food product according to the invention is a frozen confectionery product.

For the purpose of the invention the term frozen confectionery product includes milk containing frozen confections such as ice-cream, frozen yoghurt, sherbet, sorbet, ice milk and frozen custard, water-ices, granitas and frozen fruit purees. Especially preferred products of the invention are ice-cream and water-ice.

Frozen products according to the invention may be aerated. For example the level of aeration is more than 50%, more preferably more than 70%, most preferable more than 90%. Generally the level of aeration will be less than 400%, more general less than 300, most preferred less than 200%. Aeration may for example take place prior or during freezing. If the product is pre-frozen by one or more of the above describes nucleation dominated freezing processes then preferably the aeration takes place prior to (pre)-freezing.

Preferably the level of AFPs in the frozen food product of the invention is from 0.0001 to 0.5 wt% based on the final product.

The AFP for use in products of the invention can be any AFP suitable for use in food products. Examples of suitable sources of AFP are for example given in the above mentioned article of Griffith and Vanya Ewart and in our non-



prepublished patent applications PCT/EP97/03634,  
PCT/EP97/03635, PCT/EP97/03636, PCT/EP97/03637 and  
PCT/EP97/06181.

5 The AFPs can be obtained from their sources by any suitable  
process, for example the isolation processes as described  
in the above mentioned documents.

One possible source of AFP materials is fish. Examples of  
10 fish AFP materials are AFGP (for example obtainable from  
Atlantic cod, Greenland cod and Tomcod), Type I AFP (for  
example obtainable from Winter flounder, Yellowtail  
flounder, Shorthorn sculpin and Grubby sculpin), Type II  
AFP (for example obtainable from Sea raven, Smelt and  
15 Atlantic herring) and Type III AFP (for example obtainable  
from Ocean out, Atlantic wolffish, Radiated shanny, Rock  
gunnel and Laval's eelpout). A preferred example of the  
latter type is described in WP 97/02343.

20 Another possible source of AFP material are invertebrates.  
Also AFPs may be obtained from Bacteria.

A third possible source of AFP material are plants.  
Examples of plants containing AFPs are garlic-mustard, blue  
25 wood aster, spring oat, winter cress, winter canola,  
Brussels sprout, carrot, Dutchman's breeches, spurge,  
daylily, winter barley, Virginia waterleaf, narrow-leaved  
plantain, plantain, speargrass, Kentucky bluegrass, Eastern  
cottonwood, white oak, winter rye, bittersweet nightshade,  
30 potato, chickweed, dandelion, spring and winter wheat,  
triticale, periwinkle, violet and grass.

Both natural occurring species may be used or species which have been obtained through genetic modification. For example micro-organisms or plants may be genetically modified to express AFPs and the AFPs may then be used in accordance to the present invention.

Genetic manipulation techniques may be used to produce AFPs. Genetic manipulation techniques may be used to produce AFPs having at least 80%, more preferred more than 95%, most preferred 100% homology to the AFP's directly obtained from the natural sources. For the purpose of the invention these AFPs possessing this high level of homology are also embraced within the term "AFPs".

The genetic manipulation techniques may be used as follows: An appropriate host cell or organism would be transformed by a gene construct that contains the desired polypeptide. The nucleotide sequence coding for the polypeptide can be inserted into a suitable expression vector encoding the necessary elements for transcription and translation and in such a manner that they will be expressed under appropriate conditions (e.g. in proper orientation and correct reading frame and with appropriate targeting and expression sequences). The methods required to construct these expression vectors are well known to those skilled in the art.

A number of expression systems may be utilised to express the polypeptide coding sequence. These include, but are not limited to, bacteria, yeast insect cell systems, plant cell culture systems and plants all transformed with the appropriate expression vectors.

A wide variety of plants and plant cell systems can be transformed with the nucleic acid constructs of the desired polypeptides. Preferred embodiments would include, but are not limited to, maize, tomato, tobacco, carrots, strawberries, rape seed and sugar beet.

For the purpose of the invention preferred AFPs are derived from fish or plants. Especially preferred is the use of fish proteins of the type III, most preferred HPLC 12 as described in our case WO 97/02343. From plants especially the use of AFPs from carrot or grass are preferred.

For some natural sources the AFPs may consist of a mixture of two or more different AFPs.

Preferably those AFPs are chosen which have significant ice-recrystallisation inhibition properties.

Preferably AFPs in accordance to the invention provide an ice particle size upon recrystallisation as measured in accordance to the examples of less than 20  $\mu\text{m}$ , more preferred from 5 to 15  $\mu\text{m}$ .

Preferably the level of solids in the frozen food product (e.g. sugar, fat, flavouring etc.) is more than 2 wt%, more preferred from 4 to 70wt%.

For some applications it may be advantageous to include a mixture of two or more different AFPs into the food product. One reason for this can for example be that the plant source for the AFPs to be used, contains more than

one AFP and it is more convenient to add these, for example because they are both present in the AFP source to be used. Alternatively it may sometimes be desirable to add more than one AFP from different sources.

5

The invention will now be illustrated by means of the following examples.

## Example I

Method of determining whether an AFP possesses ice recrystallisation inhibition properties.

5

Recrystallisation inhibition properties can be measured using a modified "splat assay" (Knight et al, 1988). 2.5  $\mu$ l of the solution under investigation in 30% (w/w) sucrose, is transferred onto a clean, appropriately labelled, 16 mm circular coverslip. A second coverslip is placed on top of the drop of solution and the sandwich pressed together between finger and thumb. The sandwich is dropped into a bath of hexane held at  $-80^{\circ}\text{C}$  in a box of dry ice. When all sandwiches have been prepared, sandwiches are transferred from the  $-80^{\circ}\text{C}$  hexane bath to the viewing chamber containing hexane held at  $-6^{\circ}\text{C}$  using forceps pre-cooled in the dry ice. Upon transfer to  $-6^{\circ}\text{C}$ , sandwiches can be seen to change from a transparent to an opaque appearance. Images are recorded by video camera and grabbed into an image analysis system (LUCIA, Nikon) using a 20x objective. Images of each splat are recorded at time = 0 and again after 60 minutes. The size of the ice-crystals in both assays is compared by placing the slides within a temperature controlled cryostat cabinet (Bright Instrument Co Ltd, Huntington, UK). Images of the samples are transferred to a Quantimet 520 MC image analysis system (Leica, Cambridge UK) by means of a Sony monochrome CCD videocamera. Ice crystal sizing was performed by hand-drawing around ice-crystal. At least 400 crystals were sized for each sample. The ice crystal size was taken as being the longest dimension of the 2D projection of each

crystal. The average crystal size was determined as the number average of the individual crystal sizes. If the size at 30-60 minutes is similar or only moderately (less than 10%) increased compared to the size at  $t=0$ , and/or the crystal size is less than 20 micrometer, preferably from 5 to 15 micrometer this is an indication of good ice-crystal recrystallisation properties.

## Example II

A liquid premix for the preparation of ice-cream was  
5 prepared by mixing:

Ingredient	% by weight
Skimmed milk powder	10.00
sucrose	13.00
10 maltodextrine (MD40)	4.00
Locust bean gum	0.14
butter oil	8.00
monoglyceride (palmitate)	0.30
vanillin	0.01
15 AFP	0.01
water	balance

Note: AFP is carrot AFP as described in our PCT/EP97/06181

20 The composition was prefrozen to -5 C and aerated to 100%  
overrun in a traditional scraped surface heat exchanger.

The composition was further frozen in a single screw  
extruder having a barrel length of 0.75 m, a diameter of  
25 0.2 m a screw pitch of 0.135 m (2 start) and a screw  
channel depth of 15 mm.

The througput was 280 kg/hour, the inlet pressure 7 barg  
and a constant torque of the screw was 1500Nm. The output  
30 pressure was 8 barg. The screw extruder was cooled such  
that the extrusion temperature was -12 C

As a comparison (B) the same product was produced using a conventional scraped surface heat exchanger.

5 A comparison (C) the same product was produced by the above screw extruder process, whereby the AFP was omitted from the formulation.

The resulting products were stored for 3 weeks at -10 °C.

10

Composition A had a smoother and creamier texture than formulations B and C.



## Example III

A liquid premix for the preparation of ice-cream was prepared by mixing:

5		
	Ingredient	% by weight
	Skimmed milk powder	10.00
	sucrose	13.00
	maltodextrine (MD40)	4.00
10	Locust bean gum	0.14
	butter oil	8.00
	monoglyceride (palmitate)	0.30
	vanillin	0.01
	AFP (of example II)	0.01
15	water	balance

The liquid mix was continuously aerated at a throughput of 60 litres/hour using a high speed rotor/stator mixer (megatron, Kinematica AG) to an overrun of 100%. the mix temperature was 5°C and a mixer speed of 1600 rpm was employed. A pressure of 3 barg was maintained within the mixing head.

The aerated mix was then continuously spread as a 0.1 mm film on the surface of a drum freezer cooled with a methanol solution at -28 °C. The drum freezer was rotated at a rate of 1rpm. After one complete revolution the frozen film at -10 °C was removed continuously by means of a scraper blade to form frozen flakes.

30

The frozen flakes were compressed batch-wise using a piston compression device. The compressed ice cream was extruded

through a nozzle and packaged for storage. The ice crystal size distribution of the frozen material was measured as follows: by placing the coverplates smeared with the compositions to be tested within a temperature controlled cryostat cabinet (Bright Instrument Co Ltd, Huntington, UK). Images of the samples are transferred to a Quantimet 520 MC image analysis system (Leica, Cambridge UK) by means of a Sony monochrome CCD videocamera. Ice crystal sizing was performed by hand-drawing around ice-crystal. At least 400 crystals were sized for each sample. The ice crystal size was taken as being the longest dimension of the 2D projection of each crystal. The average crystal size was determined as the number average of the individual crystal sizes.

15

The average ice crystal size was 5.8 micrometer for the fresh sample with AFP and 7.2 micrometer for the fresh sample with AFP. After storage for 3 weeks at -10 C the particle size of the sample with AFP was 7.7 micrometer, without AFP 43.2 micrometer.

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#### Example IV

Example III was repeated, but now the pre-frozen flakes are fed via an hopper to a twin screw extruder (CP1050, APV) which was cooled with a methanol solution at  $-28^{\circ}\text{C}$ . Co-rotating fully intermeshing screw rotors were fitted and a rotational speed of 10 rpm was used. The ice-cream was compressed and extruded at a temperature of  $-12^{\circ}\text{C}$ .

## Example V

A liquid premix for the preparation of ice-cream was  
5 prepared by mixing:

Ingredient	% by weight
Skimmed milk powder	10.00
sucrose	13.00
10 maltodextrine (MD40)	4.00
Locust bean gum	0.14
butter oil	12.00
monoglyceride (palmitate)	0.30
vanillin	0.01
15 AFP (of example I*)	0.01
water	balance

Note: AFP is AFP of HPLC-12 as described in our WO  
97/02343.

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The mix was aerated to 100% overrun as in example III. The  
aerated mix was frozen in the form of 10 mm diameter  
pellets using a cryogenic freezing unit (British Oxygen  
Company). The freezing surface consisted of a rotating  
25 horizontal turntable, which was cooled using liquid  
nitrogen to a temperature of -100 °C. The air above the  
freezing turntable was also cooled to a temperature of -120  
°C. The turntable was rotated at 5rpm. After a single  
rotation the frozen pellets were swept off the freezing  
30 surface and collected.

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The frozen pellets were then fed into a screw extruder under the same conditions as in example IV.

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Claims

1. A process for the manufacture of a frozen food product comprising AFPs, wherein the freezing process involves the use of a screw extruder or a compactor.
2. A process according to claim 1, wherein the frozen food product is a frozen confectionery product.
3. A process according to claim 1 or 2, wherein a screw extruder is used and the extrusion temperature of the frozen food product is less than -8 °C.
4. A process according to one or more of the preceding claims, further comprising a pre-freezing step.
5. A process according to claim 4, wherein the pre-freezing involves a nucleation dominated freezing process.
6. A process according to claim 5, wherein the pre-freezing involves the drumfreezing of the product.
7. A process according to any of the preceding claims, whereby the product is not subjected to a hardening step.
8. A process according to any of the preceding claims wherein the frozen product is an ice-cream product and the production thereof comprises the steps of:
  - (a) extruding block of ice-cream;
  - (b) insertion of a stick into the block of ice-cream;
  - (c) coating of the block of ice-cream.

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9. A non-particulate frozen food product comprising AFPs, said product having an average ice crystal size of 0.01 to 20 micrometer, wherein said crystal size is maintained between 0.01 and 20 micrometer upon storage for 3 weeks at -10 °C.

Abstract

A process for the manufacture of a frozen food product comprising AFPs, wherein the freezing process involves the use of a screw extruder or compactor.